CONCISE REPORT

The Variation of Hematopoietic Stem Cell Self-Renewal Capacity as a Function of Age: Further Evidence for Heterogeneity of the Stem Cell Compartment

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There is substantial evidence for limited proliferative capacity of the hematopoietic progenitors. Hematopoiesis in mice is maintained by primitive stem cells that are assayed by their ability to form spleen colonies (CFU-S). To measure the average proliferative capacity of these cells (CFU-S), bone marrow containing 50–150 CFU-S is injected into lethally irradiated mice, and 14 days later, the number present in the recipient marrow is measured. In fetal life between 13 and 16 days of gestation, the hematopoietic stem cells are primarily localized in the liver. There is a decrease in the average proliferative capacity of these stem cells as they progress from fetal life into adulthood. This proliferative capacity of these significantly increases in late adulthood. However, if one scores maximum self-renewal or proliferative capacity by another assay consisting of the serial transplantation of marrow at 14-day intervals, this capacity is unchanged with age. These observations can be explained by considering the stem cell compartment as a continuum of cells whose self-renewal capacity varies inversely with their divisional history. All stem cells participate in repopulation when the pool needs rapid expansion (fetal life). During steady-state repopulation (adult life), clones are selected on the basis of their divisional history, those with the greatest proliferative history being selected. Thus, the average proliferative capacity of the remaining stem cells will increase as the adult animal ages, since stem cells of low proliferative potential have been consumed. The maximal self-renewal capacity will remain unchanged, since this measures the spared earliest cells with the highest proliferative capacity.

The mechanisms by which the hematopoietic stem cell compartment maintains active cell production throughout the life of the animal is unknown. Data from Hayflick suggest that normal diploid cell proliferation in vitro is limited.1 Whether this observation relates to any or all in vivo circumstances is unclear.2 3 We, as well as others, demonstrated that the marrow stem cell compartment has a limited proliferative capacity,4 6 and this proliferative potential is further limited by cytotoxic agents.7 9 Kay has reconciled the conflict between limited proliferative capacity of stem cells for self-renewal and their extensive requirement for mature progeny production throughout life by suggesting that all stem cells do not participate in cell production under normal circumstances.10 Thus, all the progeny of a few stem cells maintain the organism until their proliferative capacity is exhausted and then they are replaced by new stem cells (clonal succession). Most of the stem cells would not contribute to normal hematopoiesis and would be proliferatively inactive. The notion that the most primitive stem cells form a reserve compartment has been suggested as a useful mechanism to protect against the incorporation of genetic error.11

Rosendaal et al. have suggested that there is a hierarchy of stem cells in vivo based on their previous proliferative history.12 In this report, we investigated whether stem cell proliferative potential decreases in vivo from fetal to adult life and whether there is a hierarchy in determining how clones are recruited into proliferation. If this hypothesis is true, those stem cells with the greatest proliferative history and lowest remaining self-renewal capacity should be preferentially selected for repopulation during aging. This should result in the average self-renewal capacity increasing with age. However, the maximum self-renewal capacity of the stem cell compartment should not increase, since the earliest cells would be spared.

MATERIALS AND METHODS

The murine spleen colony (CFU-S) assay of Till and McCulloch13 was used to determine the number of CFU-S present in either C3H/HeJ male bone marrow or 13–16-day fetal liver. At least 8 animals were used as recipients for each CFU-S determination in all of the assays described. Donor hematopoietic tissue was pooled from C3H/HeJ male bone marrow of 3 mice or from 16 fetal livers. All injected cells were dispersed in Tyrode solution and kept on ice for less than 3 hr prior to injection.8

To measure the average self-renewal capacity of the hematopoietic stem cell compartment, aliquots of femoral marrow cells or 13–16-day-old fetal liver cells containing 50–150 CFU-S were injected into lethally irradiated (1250 rad) recipient C3H/HeJ male mice 8–12 wk of age. Fourteen days later, the number of CFU-S in the recipient femoral bone marrow was measured. The ratio of the number of CFU-S produced in 14 days as compared to the number of CFU-S injected is referred to as R.14 It may be calculated as follows:
$R_i = K \frac{S_n}{S_i}$ where $K$ is a constant $= 0.1$ (the proportion of marrow normally contained in one femur), $S_i$ = CFU-S injected, and $S_n$ = CFU-S produced. The more CFU-S produced, the higher $R_i$ will be and the greater the average proliferative capacity of the injected CFU-S.

To measure maximum proliferative capacity, cells were serially transplanted at 14-day intervals. One million marrow or fetal liver cells were transplanted initially. After 14 days the lethally irradiated recipients were sacrificed and the marrow removed. Thereafter, marrow transplantation was repeated at 14-day intervals until there were insufficient cells to deliver $10^6$ cells to at least 8 recipients or until all the animals died prior to day 14. The maximum total transplantation time would be 35 days. Complete transfers but the last surviving animal died by day 7 of the third transfer, the total transplantation time would be 35 days. The maximum total transplantation time would be 35 days. The higher the total transplantation time, the greater the average proliferative capacity of the injected CFU-S.

Determinations of the average proliferative capacity of the stem cell compartment were made in fetal life and throughout adult life. The data are shown in Fig. 1. The $R_i$ is highest in fetal life and then decreases in the young adult but rises again as the mouse ages. The $R_i$ determinations for fetal liver ($R_i = 60.1 \pm 9.6$) are significantly higher than the 10-wk-old bone marrow ($p < 0.01$). The positive slope seen for the adult bone marrow determinations is significantly different than zero ($p < 0.001$) as determined by linear regression despite the large scatter of data points. Because of this scatter, it is not possible to determine the exact shape of the curve and thus a straight line is assumed. Other simple or compound shapes are possible. Logarithmic plotting gives a straight line with a positive slope.

Grouping the data points into 10–20-wk intervals suggests a 2-phase curve that rises abruptly at 40–60 wk of age. Regardless of how the data are plotted, it is clear that the marrow $R_i$ is lowest during young adulthood. There may be some defect in the maximum proliferative potential of old marrow from young donors. Growth in continuous long term culture may be more stressful or more sensitive as an assay than serial transplantation.

To determine the maximum proliferative capacity of the stem cell compartment as it progresses from fetal life to the aged mouse, serial fetal liver or marrow transplantation was performed. As can be seen from Fig. 2, this capacity does not appear to change significantly, although a slightly negative slope with increasing adult age is noted. At least 3 serial transfers were attempted for each point on the curve. The transfer capacity of fetal liver (40.8 ± 1.2 days) is not statistically different than the young (10–45 wk) adult (39.1 ± 1.02 days) marrow.

**DISCUSSION**

These data suggest that the bone marrow stem cell compartment of the mouse is heterogeneous with respect to self-renewal. There appear to be different patterns of hematopoietic proliferation in utero and in adult cell renewal systems. First there is a uniform proliferative participation of stem cells in order to establish the compartment. This is followed by a hierarchical clonal participation of stem cells to maintain the steady state. Because all stem cells prolifer-
ate during early fetal life, initially there should be a decrease in the self-renewal capacity of the pool as measured both by the average self-renewal ($R_s$) (Fig. 1) and the maximum self-renewal (as measured by serial transplantability) (Fig. 2). Although a decrease in $R_s$ was seen, we did not observe a significant decrease in serial transfer from fetal hematopoiesis (13–16 days) into young adulthood. However, Metcalf and Moore, using earlier hematopoietic tissue from the yolk sac and 10-day fetal liver, did show an increased capacity in serial transfer (< 50 days) when comparing fetal to adult hematopoiesis.\(^{16}\) Perhaps older (13–16 days) fetal hematopoietic tissue represents a transition state in which uniform proliferative participation is coming to an end. Thus, the most primitive stem cells would have become proliferatively quiescent but unchanged with respect to maximum proliferative capacity. Our attempts at studying yolk sack tissue were unsuccessful, since the recipient mice did not survive the injection process, possibly the result of pulmonary fat emboli. Since the $R_s$ decreases from the 13–16-day-old fetus to the young adult, while maximum proliferative capacity does not, this fall in $R_s$ may be caused by an increase in the number of less potent stem cells. During adult life, hierarchical clonal succession predominates, so that the less potent stem cells are preferentially selected for amplification and maintenance of hematopoiesis, while the earlier stem cells are spared. This results in the $R_s$ increasing during adult life without an associated change in maximum self-renewal as measured by serial transplantation. There may be some defect in the maximum proliferative potential of old marrow from young donors.\(^{17}\) Growth in continuous long term culture may be more stressful or more sensitive as an assay than serial transplantation.

There may be objections to the $R_s$ as a measure of stem cell proliferative capacity, since it does not consider the complete regrowth curve of the injected CFU-S. According to extensive studies in our lab, cytotoxic agents that cause diminution in the $R_s$ for as long as 650 days after drug injection also cause prolonged defects in marrow proliferative capacity.\(^{8,9}\) Those drugs that do not decrease $R_s$ do not affect marrow proliferative capacity. Thus, the $R_s$ measured at day 14 appears to be a useful indicator of proliferative capacity.

The data reported here provide evidence to support both heterogeneity and limitation of the proliferative capacity of the hematopoietic stem cell compartment. The dual mechanisms of uniform proliferation and clonal succession allow for both the maximum response required by the severe proliferative demands of fetal life, while conserving the limited proliferative capacity of the stem cells during the steady state and following lesser proliferative stresses.

**REFERENCES**


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